Molecular Biology

PAROMOYCIN-DEPENDENCE IN *SACCHAROMYCES CEREVISIAE*

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Wild-type strains of the eukaryote, *Saccharomyces cerevisiae* (baker's yeast), are not noticeably affected by the aminoglycoside antibiotics streptomycin or paromomycin. In contrast, wild-type prokaryotes, including *Escherichia coli* and other bacteria, are killed by these antibiotics, which interact with components of the small ribosomal subunit and adversely affect the accuracy of protein synthesis. However, certain mutant strains of bacteria exhibit streptomycin -resistance or -dependence due to specific changes in key ribosomal proteins. One of the proteins often altered in such mutant strains of *E. coli* is the small subunit ribosomal protein S12. The purpose of this investigation is to determine if analogous antibiotic-dependence can be generated in the simple eukaryote *S. cerevisiae*. Herein, we describe the production of several yeast isolates that exhibit a strict dependence on paromomycin for growth. To our knowledge, such aminoglycoside-dependence has not been previously described in a eukaryote.

Initially, we hypothesized that previous attempts to produce antibiotic-dependent yeast were not successful because the yeast homologue of bacterial ribosomal protein S12, Rps23, is produced from duplicate genes (*RPS23A* and *RPS23B*) in haploid yeast strains. And the likelihood of randomly inducing specific genetic changes in both genes simultaneously is very improbable. Therefore, we used ethylmethanesulfonate (EMS) to randomly mutagenize a haploid yeast strain that harbors a disrupted *RPS23B* gene. In this strain, all of the Rps23 incorporated into ribosomes is derived from the *RPS23A* gene. EMS-treated cells were enriched for paromomycin-dependent mutants using nystatin to select against cells exhibiting paromomycin-independent growth. Six paromomycin-dependent isolates were ultimately identified using this procedure. It is notable that streptomycin does not support the growth of these mutants. The paromomycin-dependence is recessive in each isolate and significant affects on temperature-sensitivity are also noted. We suspect that the mutations responsible for this phenotype occur in the *RPS23A* gene, although we continue working to identify the specific genetic changes responsible for this novel aminoglycoside-dependent phenotype.

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